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Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems

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Abstract

The presence of highly unsaturated fatty acids is a principal factor which determines the food value of *Artemia* nauplii for marine shrimp and fish larvae. In an attempt to explain the high variation in essential fatty acid content in *Artemia* from different sources we have studied the effect of diet on the fatty acid profile of Artemia offspring (ovi- or ovoviviparous) produced in controlled culture systems. The parental populations tested originated from Lavalduc (France) and Great Salt Lake (Utah, USA); they were fed different diets consisting of dried Spirulina and/or defatted rice bran, eventually coated with cod liver oil. The analytical data indicate that the fatty acid profile of the *Artemia* offspring reflects the composition of the diet fed to the parental brine shrimp regardless of the strain used. Moreover, the level of ω3 highly unsaturated fatty acids (ω3-HUFA) in the cysts and nauplii can be significantly increased by feeding the parental stock with ω3-HUFA-fortified diets. From this study we can conclude that the natural production of *Artemia* cysts with a high ω3-HUFA content will be limited to those biotopes where natural or man-managed conditions enhance the dominant presence of a ω3-HUFA-rich diet.

KEYWORDS: *Artemia*, Nutrition, Fatty acids, Cysts, Nauplii.

Introduction

A very important factor determining the dietary value of *Artemia* as a food source for marine fish- and crustacean larvae is the level of the essential fatty acids eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) (Watanabe et al., 1980; Léger et al., 1985, 1986). Analyses of the highly unsaturated fatty acid (HUFA) content of various *Artemia* samples (see review by Léger et al., 1986) revealed a distinct variability among different strains and within the strain, both between years as within 1 year. Contrary to the Great Salt Lake strain, most populations have particularly variable levels of 20:5ω3 (e.g. San Francisco Bay (California, USA), Macau (Brasil), People's Rep. China). Inoculation experiments with the San Francisco Bay strain in various salt ponds in Asia resulted also in cyst products with varying fatty acid profiles (Vos et al., 1984). Since the algal composition in these extensive productions varied due to different management and climatological conditions, and since several authors already demonstrated that zooplankton organisms, including *Artemia*, mainly reflect the fatty acid pattern of their food (Hinchcliffe and Riley, 1972; review by Léger et al., 1986), it is very likely that a correlation may exist between the type of food ingested by the cyst-producing females and the fatty acid profile of their offspring.

In an attempt to better explain the high variation in essential fatty acid content in *Artemia* cysts from various batches we have studied the effect of diet composition fed to parental populations in intensive production systems on the fatty acid profile of their offspring.

Materials and methods

Two batches of *Artemia* cysts from different

Table I. Summary of specific culture conditions for the production of *Artemia* offspring

Culture conditions	Food	Salinity (‰)	Animal density ($ind.l^{-1}$)
Cyst production unit			
- Lavalduc - RB	Micronized rice bran	50	10 000
- Lavalduc - SPIR	Micronized rice bran, spray-dried *Spirulina* (3:1)	50	10 000
- Lavalduc - RBA	Micronized defatted rice bran coated with 6% cod liver oil	75	10 000
- Great Salt Lake - RB	Micronized rice bran	50	10 000
Nauplii production unit			
- Great Salt Lake - RB	Micronized rice bran	35	5 000
- Great Salt Lake - SPIR	Micronized rice bran dried *Spirulina* (3:1)	35	5 000

species (*A. parthenogenetica*, *A. franciscana*) were selected as an inoculum for the reproduction tests; i.e. Lavalduc, France (LVD, batch 256-1979), respectively Great Salt Lake, Utah, USA (GSL, batch 185-0). Culture and induction techniques for the production of only one type of offspring (either cysts or nauplii) are described in detail by Lavens and Sorgeloos (1984, 1987). The specific culture conditions and experimental set-up are summarized in Table I. Rice bran (RBA) enriched with fish oil was prepared by coating defatted rice bran (multiple petroleum ether extraction) with cod liver oil dissolved in aceton. The solvent was then distilled under lowered pressure.

Cysts collected from the cyst production unit were separated from debris using the biphase flotation technique as described by Sorgeloos et al. (1986). Fatty acid analyses were carried out on ovoviviparously produced nauplii, collected within an interval of maximum 3h after deposition. In the cyst producing populations fatty acid analyses were performed on decapsulated cysts (Bruggeman et al., 1980) and not on the hatched nauplii, i.e. hatching performance of lab-produced cysts can be very variable (hatching percentage and rate: Lavens et al., 1986) as a result of which the available nauplii might not be representative for all produced cysts or might not belong to the same stage (instar I). Instar I nauplii, hatched at stage T90 (Vanhaecke and Sorgeloos, 1983) from the parental batch of cysts were also analyzed for comparison.

Fatty acid profiles were determined by capillary gas chromatography. Decapsulated cysts or nauplii were homogenized with an ultrasonic homogenizer (Sonifier B12). Lipid extraction, saponification and esterification was done according to the procedure described by Schauer and Simpson (1978). Fatty acid methyl esters (FAME) were injected on a capillary column (25m fused silica, ID: 0.32mm, liquid phase: SILAR 10C, film thickness: 0.3m) installed in a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were as follows : solid injector; carrier gas: hydrogen; flow rate 1.9ml.min^{-1}; FID; oven temperature program: 154°C to 200°C at 2°C.min^{-1}. Peak identifications and quantification was done with a calibrated plotter-integrator (Hewlett-Packard 3390 A) and reference standards. The results are presented as area-percent FAME composition and as mg FAME.g^{-1} dry weight.

Results and discussion

Fatty acid analyses reveal a clear difference in profile between the parental material and the F1-produced cysts (Table II), respectively ovoviviparous nauplii (Table III). When comparing these data with the analyses of the different feeds fed to the *Artemia* cultures (Table IV) it can be concluded that the fatty acid content of *Artemia* offspring reflects the profile in the feed fed to the parental population.

Micronized rice bran contains high amounts of 16:0, 18:1, and 18:2. These fatty acids are also abundantly present in either type of Lavalduc and Great Salt Lake *Artemia* offspring produced on this diet. partial substitution of the rice bran with *Spirulina* (rich in 16:1 and 18:3ω6) consequently gives much higher levels of these fatty acids in Lavalduc cysts and Great Salt Lake ovoviviparous nauplii.

Moreover, both diets are deficient in HUFA's which is again reflected in the profiles of the *Artemia* offspring. Incorporation of these HUFA's in the parental diet by coating the rice bran particles with cod liver oil significantly improves the HUFA-values in the cysts consequently produced: e.g. high levels of 20:5ω3 and some 22:6ω3. Important to notice is that the ω3-HUFA content in the laboratory produced Lavalduc cysts could be increased to higher levels than in the natural cysts by feeding ω3-HUFA fortified diets to the parental population; i.e. 8.7 versus 6.2mg.g^{-1} dry weight. Although no experiments have been carried out so far with the emulsified ω3-HUFA concentrate (Léger et al., 1987), we can expect that by this method even higher levels of the essential fatty acids 20:5ω3 and 22:6ω3 could be incorporated in the offspring of *Artemia* independently of the strain used. It is also remarkable that there is no inverse relationship between the concentration of 18:3ω3 and 20:5ω3, as was demonstrated for natural batches of *Artemia* cysts (Léger et al., 1986).

The fact that parental diet composition interferes with the nutritional quality, i.e. with the essential fatty acid (EFA) content of the offspring produced, is further supported by some

Table II. Data on qualitative and quantitative fatty acid composition (FAME) of parental *Artemia* cysts and their encysted offspring produced with different diets. (a) area % fatty acid methyl esters (FAME); (b) mg FAME per gram dry weight; (tr) trace

FAME	LVD 256		LVD-RB		LVD-RBA		LVD-SPIR		GSL185		GSL-RB	
	a	b	a	b	a	b	a	b	a	b	a	b
14:0	1.7	2.0			0.8	1.0	0.6		1.0	1.8	1.0	0.8
14:1	1.4	1.6	0.3	0.4	1.0	1.1	1.2		0.3	2.0	0.3	0.3
14:2									0.8	1.5	0.1	0.1
15:0	0.5	0.5	0.2	0.2	tr	tr	0.1		0.8	1.5	0.1	0.1
15:1	tr	tr			0.1	0.1	0.1		0.3	0.5	0.2	0.2
16:0	14.5	16.5	12.0	12.5	7.3	8.4	10.9		14.1	25.7	10.6	9.1
16:1ω7	8.6	9.8	2.5	2.6	10.4	11.8	4.8		4.4	8.1	2.2	1.9
16:1ω9	tr	tr										
16:2			0.8	0.8	0.7	0.8	0.3		1.0	1.8	tr	tr
16:3	2.2	2.5	0.2	0.2	0.4	0.4	1.0		1.6	2.9	0.7	0.6
17:0	0.6	0.7	0.3	0.3					0.8	1.5	0.4	0.3
18:0	3.5	4.0	3.2	3.3	1.2	1.4	2.8		3.6	6.3	3.3	2.9
18:1ω7	6.4	7.3			45.5	51.9	36.3		27.3	49.8	41.6	35.8
18:1ω9	18.3	20.9	40.3	41.9								
18:2ω6	**6.4**	**7.3**	**37.5**	**39.0**	**22.4**	**25.5**	**36.0**		**6.1**	**11.1**	**36.2**	**31.2**
18:3ω3	**20.0**	**22.7**	**1.5**	**1.6**	**1.3**	**1.5**	**2.7**		**27.1**	**49.4**	**1.3**	**1.1**
18:3ω6	0.9	1.0					1.7			tr	tr	
18:4ω3	2.1	2.3			tr	tr	0.5		3.3	6.0	0.1	0.1
19:0	0.5	0.6									tr	tr
20:0			0.1	0.1							0.3	0.2
20:1ω9	3.2	3.8	0.7	0.8	0.6	0.7			0.4	0.8	0.5	0.4
20:2ω6												

Table II. Continued

FAME	LVD 256		LVD-RB		LVD-RBA		LVD-SPIR		GSL185		GSL-RB	
	a	b	a	b	a	b	a	b	a	b	a	b
20:3ω3							0.1		0.4	0.7		
20:3ω6											0.1	0.1
20:4ω3	**1.2**	**1.4**	**0.1**	**0.1**	**0.2**	**0.2**	**0.4**		**0.6**	**1.1**	**0.1**	**0.1**
20:4ω6												
20:5ω3	**5.4**	**6.2**	**0.4**	**0.4**	**7.7**	**8.7**	**0.2**		**2.1**	**3.9**	**0.4**	**0.3**
21:5	0.2	0.3					tr					
22:1							0.1				0.3	0.2
22:3							tr		0.2	0.4		
22:4ω3	tr	tr							tr	tr		
22:4ω6												
22:5ω3									tr	tr		
22:5ω6												
22:6ω3	**0.5**	**0.6**			**0.6**	**0.7**			**tr**	**tr**		
24:1												
Non identified peaks	1.9								3.0		0.2	
Σω 3 HUFA	7.1	8.2	0.5	0.5	8.5	9.6	0.6		3.3	6.1	0.5	0.4
Total lipids (mg total lipid/g dry weight)		17.3				20.9				17.4		17.4

Table III. Data on qualitative and quantitative fatty acid composition (FAME) of parental Great Salt Lake cysts and their ovoviviparous offspring produced with different diets. See legend Tables II and IV

FAME	GSL 185		GSL-RB-N		GSL-SPIR-N	
	a	b	a	b	a	b
14:0	1.1	1.7	1.6	2.2	2.5	3.9
14:1	0.9	1.5	0.9	1.3	1.1	1.7
14:2	0.3	0.4			0.3	0.5
15:0	0.3	0.5	0.6	0.8	1.3	2.0
15:1	0.9	1.4	0.6	0.8	tr	tr
16:0	12.3	19.7	12.1	16.3	16.0	25.3
16:1ω7	4.4	7.0	4.8	6.4	6.1	9.7
16:1ω9	0.4	0.7	1.0	1.4	0.9	1.4
16:2			0.1	0.2		
16:3	1.2	2.0	0.7	1.0	1.2	2.0
17:0	0.4	0.7	0.5	0.6	1.5	2.3
18:0	3.8	6.1	4.5	6.1	7.1	11.3
18:1ω7	10.2	16.4	35.6	47.7	26.5	47.9
18:1ω9	17.1	27.4				
18:2ω6	**7.6**	**12.1**	**27.8**	**37.2**	**15.2**	**24.0**
18:3ω3	**29.1**	**46.7**	**1.9**	**2.5**	**1.3**	**2.1**
18:3ω6	0.1	0.1	0.2	0.2	6.7	4.2
18:4ω3	3.8	6.0	tr	tr		
19:0	0.6	0.9	tr	tr		
20:0	0.2	0.3	tr	tr	1.6	2.6
20:1ω9	0.1	0.1	0.5	0.7	1.4	0.9
20:2ω6						
20:3ω3	0.1	0.1			0.1	0.2
20:3ω6	0.2	0.3	1.0	1.3	0.3	0.5
20:4ω3	**0.4**	**0.6**	**0.8**	**1.1**	**1.6**	**2.5**
20:4ω6	0.2	0.3				
20:5ω3	**2.6**	**4.2**	**0.5**	**0.7**	**0.8**	1.3
21:5	0.4	0.6				
22:1	0.5	0.7			0.7	1.1
22:3ω3						
22:4ω3	0.1	0.1				
22:4ω6						
22:5ω3	tr	tr				
22:5ω6	tr	tr				
22:6ω3						
24:1						
Non identified peaks	0.7		4.3		58	
Σω3 HUFA	3.2	5.0	1.3	1.8	2.5	4.0
mg total lipid per g dry weight		27.0				

Table IV. Data on qualitative and quantitative fatty acid composition (expressed in fatty acid methyl esters FAME) of the experimental diets fed to the *Artemia* cultures. See legend Tables I and II

FAME	Rice bran RB		HUFA-enriched rice bran (RBA)		3:1 mixed diet of rice bran and *Spirulina* (SPIR)	
	a	b	a	b	a	b
14:0	0.9	0.3	2.4	1.5	0.6	0.2
14:1	0.1	0.1	0.1	0.1	0.1	0.1
14:2	-	-	0.1	0.1		
15:0	0.2	0.1	0.3	0.2	0.9	0.3
15:1	0.2	0.1	0.1	0.1	1.5	0.4
16:0	21.8	6.4	14.4	9.0	25.3	7.1
16:1ω7	0.6	0.2	5.3	3.3	2.9	0.8
16:1ω9	0.3	0.1	0.3	0.2	0.2	0.1
16:2	0.1	0.1	0.1	0.1	0.1	0.1
16:3			0.2	0.1	0.1	0.1
17:0			0.3	0.2	0.2	0.1
18:0	3.5	1.0	2.9	1.8	3.1	0.9
18:1ω7	39.9	11.4	28.5	17.9	29.9	8.4
18:1ω9						
18:2ω6	27.5	8.0	10.5	6.6	24.3	6.8
18:3ω3	**1.5**	**0.4**	**0.7**	**0.5**	**1.4**	**0.4**
18:3ω6	**0.1**	**0.1**	**0.1**	**0.1**	**5.0**	**1.4**
18:4ω3	0.2	0.1	1.4	0.9	0.2	0.1
19:0					0.1	0.1
20:0	0.8	0.2	0.4	0.2	0.2	0.1
20:1ω9	0.7	0.2	5.2	3.3	0.4	0.1
20:2ω6						
20:3ω3			0.1	0.1		
20:3ω6	0.5	0.2	0.2	0.2	0.4	0.1
20:4ω3	**0.2**	**0.1**	**0.1**	**0.1**	**0.2**	**0.1**
20:4ω6	0.1	0.1				
20:5ω3	**0.7**	**0.3**	**6.7**	**4.2**	**0.7**	**0.2**
21:5			0.5	0.3	0.1	0.1
22:1			5.1	3.2		
22:3ω3			tr	tr		
22:4ω3			0.2	0.1		
22:4ω6			0.3	0.2		
22:5ω3			0.5	0.3	0.2	0.1
22:5ω6			tr			
22:6ω3			7.2	4.6	0.1	0.1
24:1					0.7	0.2
Non identified peaks	0.1	0.1	5.7	3.5	1.1	0.5
Σω3 HUFA	0.9	0.4	14.6	9.4	1.2	0.5
Total lipids (mg total lipid per g dry weight)		5.8		6.0		6.8

extensive *Artemia* trials in Southeast Asia (Vos et al., 1984); i.e. analyses of cysts produced in ponds fertilized with anorganic fertilizers showed low HUFA levels whereas cysts from ponds treated with organic fertilizers such as poultry manure, or using intake water from mangroves, contained considerable levels of HUFA's. The control of algal composition (species diversity as well as HUFA-content) might be feasible in small production ponds; it is, however, not conceivable in large solar salt operations (e.g. San Francisco Bay, California, USA; Macau, Brazil) nor in the big salt lakes such as the Great Salt lake (Utah, USA). The nutritional quality of the cysts produced in the latter areas can therefore never be controlled: e.g. for years the dominant algal species in Great Salt Lake has been *Dunaliella*, poor in ω3-HUFA (Scott and Middleton, 1979), hence the consistently low 20:5ω3 levels in GSL cysts. The variability in the essential fatty acid content in cysts collected from solar salt operations may be explained by the wide range in ecological conditions (e.g. algal species composition) in the evaporation ponds maintained at various salinities (Carpelan, 1957; Haynes and Hammer, 1978), and/or the variability in fatty acid profile within the same algal species due to varying abiotic circumstances (Moal et al., 1978; Scott and Middleton, 1979; Enright, 1984).

The opportunistic dependency on nature for the production of high HUFA-quality *Artemia* cysts is risky and may result in temporary shortages which can only partially be overcome by naupliar HUFA-enrichment since the larger prey size may be a limiting factor. (Léger et al., 1987). The alternatives given in order of increased guarantee for harvest of quality cysts are small man-managed pond production (Sorgeloos et al., 1986) and intensive culture (Lavens and Sorgeloos, 1984); however, the latter technique may never be cost-effective.

Conclusions

- The results clearly demonstrate that the fatty acid profile of either type of *Artemia* offspring reflects the composition of the diet fed to the parental brine shrimp population, regardless of the strain used.

- Essential fatty acid content in the cysts and/or nauplii can significantly be increased by feeding the parental stock with ω3-HUFA fortified diets. In this way HUFA-levels higher than those found in cysts from natural populations can be obtained.

- The results from this study suggest that the variation in HUFA-content among cyst batches, even within the same strain, are due to spatial and/or periodical variations in the composition of microalgal species available as food for *Artemia* in the production habitats. As a result the natural production of *Artemia* cysts with high ω3-HUFA contents will be limited to those sites where natural or man-managed conditions enhance the dominant presence of a ω3-HUFA-rich diet.

- Provided its upscaling is successful the laboratory technique for controlled cyst production opens interesting perspectives for the production of "standard *Artemia* cysts" of reproducible high (even better than natural) HUFA quality. Those cysts would be a very valuable alternative for Reference Artemia Cysts as standard intercalibration material in culture tests with different predators (Sorgeloos, 1980). Furthermore cysts could be produced with specific HUFA composition (both qualitative and quantitative; e.g. cysts with a marine copepod-like HUFA composition) and be used as very valuable test-diets in the study of HUFA-requirements in larval fish and shrimp.

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